SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

AD-A204 016

DTIC ELECTE OCT 05 1988 D

DTIC FILE COPY

NA	1b RESTRICTIVE MARKINGS
NA	3. DISTRIBUTION / AVAILABILITY OF REPORT Unlimited
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NA	5. MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a. NAME OF PERFORMING ORGANIZATION University of California Santa Barbara CA 93106	6b. OFFICE SYMBOL (If applicable) NA
7a. NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c. ADDRESS (City, State, and ZIP Code) Marine Science Institute University of California Santa Barbara, CA 93106	7b. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, Virginia 22217-5000
8a. NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research	8b. OFFICE SYMBOL (If applicable) ONR
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0177	
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification)
Host-symbiont interactions between a marine mussel and methanotrophic bacterial symbionts

12. PERSONAL AUTHOR(S)
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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 10/1/87 TO 9/30/88	88/10/1	6

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Symbiosis/Methanotroph/Mussel/Genetic Integration/ Host-symbiont interactions

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Our goal is to delineate the interactions between a mussel and its methanotrophic symbionts. In the past year we have: 1) Demonstrated shell growth with methane as sole carbon and energy source. 2) Demonstrated that methane alone is not sufficient for tissue maintenance over several months. 3) Demonstrated and quantified both clearance and assimilation of particulates by the mussel. 4) Developed a protocol for the purification of the bacterial symbionts. 5) Collected (by submersible) and shipped back to our laboratory sufficient mussels for next year's studies. 6) Expanded our maintenance facilities for the mussels. 7) Measured methane, thiosulfate and sulfide in freshly collected mussel tissues, and in interstitial water from their environment. 8) Identified N_2 gas as a source of nitrogen for the intact symbiosis and begun investigations into other possible sources (NH_4, NO_3 and dissolved amino acids). 9) Begun studies of organic carbon transfer between the symbionts and the host mussel.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	(U)

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Dr. M. Marron	202 696-4760	ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

88 10 4 106

Date: 10/1/88

ANNUAL REPORT ON CONTRACT N00014-88-K-0177 R&T CODE 4412029

PRINCIPLE INVESTIGATORS: Drs. Charles Fisher and James Childress

CONTRACTOR: Marine Science Institute, University of California, Santa Barbara.

CONTRACT TITLE: Host-Symbiont Interactions Between a Marine Mussel and Methanotrophic Bacterial Endosymbionts

START DATE: 1 October 1987

RESEARCH OBJECTIVE: To delineate the interactions between a newly discovered mussel and its methanotrophic symbionts in order to reach a more complete understanding of the intact symbiosis.

PROGRESS (Year 1):

Since our start date of Oct. 1, 1987 we have made considerable progress in determining the importance of various food and energy sources to this symbiosis. Work begun in July of last year has demonstrated that the mussels can grow with methane as the sole carbon and energy source. However, in a parallel study we have found that although the shell is growing in response to methane, the mussels' soft tissues are being depleted under these experimental conditions and this is reflected in the lower Condition Index of the mussels. This study also demonstrates that neither unicellular algae (as a food source) nor thiosulfate (as a symbiont energy source) are by themselves sufficient for either shell or soft tissue growth in this mussel. (Thiosulfate was suggested as an additional possible energy source for the symbionts by some of our earlier work, Fisher et al., 1987).

We have, in a separate study, been directly testing the seep mussels ability to filter feed on particulates (both algae and bacteria). This study is now complete and our data indicates that this mussel can clear both algae and bacteria from the surrounding water and assimilates a significant portion of the particulates it clears. In comparison to Mytilus edulis (the common intertidal mussel) the seep mussels clearance rate for algae is lower, but the clearance rate for bacteria is about the same (see Figure). This is

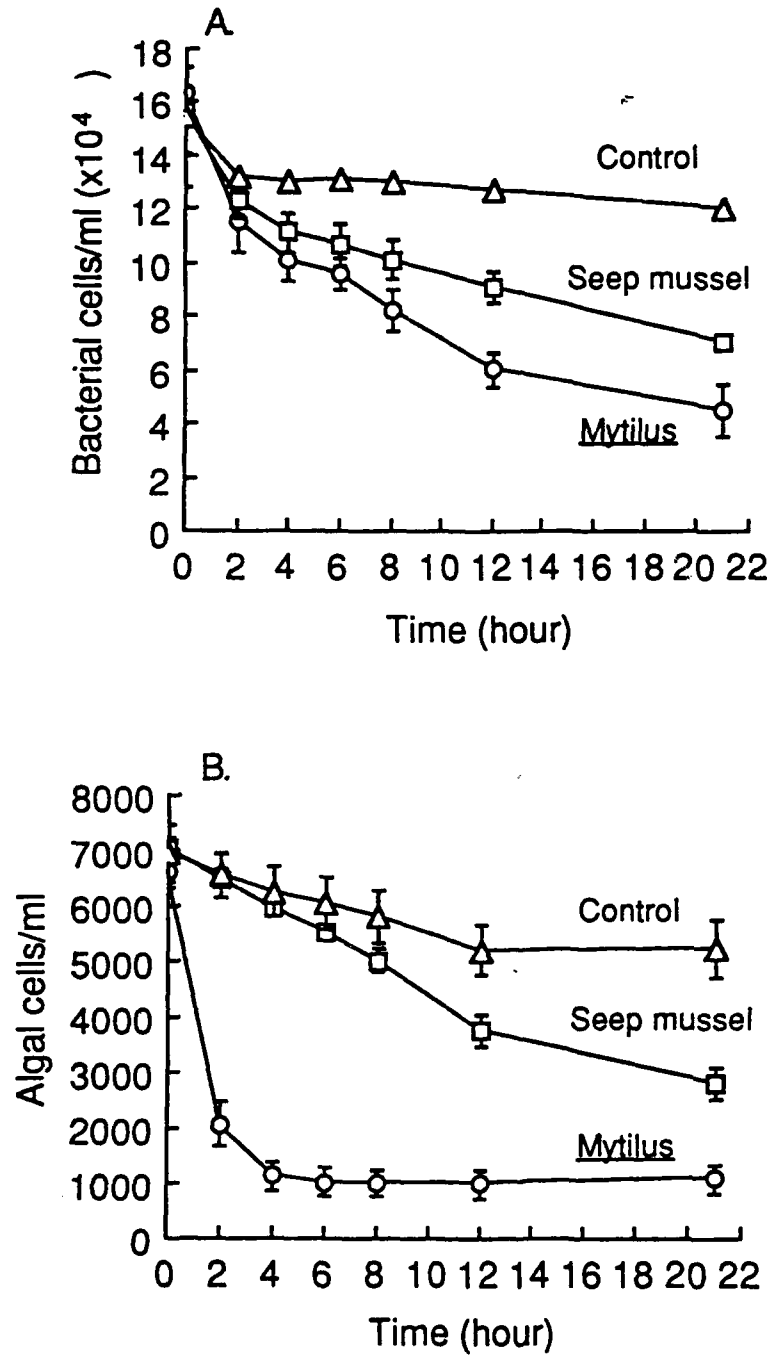

Figure 1: Clearance of bacteria (A) and algae (B) by the seep mussel and Mytilus edulis.

significant because the seep mussels ability to filter-feed had been questioned on anatomical grounds (Pers. Comm., Dr. Ruth Turner, Harvard Museum of Comp. Zool.).

A pulse-chase series of experiments have been conducted with the seep mussels in ^{14}C labeled methane. The experimental animals have been fixed and embedded in preparation for autoradiographic analysis. A parallel series of experiments will be conducted in November with larger animals and these will be dissected and prepared for scintillation counting of total incorporated organic ^{14}C. These experiments will indicate the pathway of carbon transfer between the symbiotic partners.

We have developed a method for purifying the bacterial symbionts from the mussels' gill tissue. In this method we combine differential centrifugation and filtration through a series of Nitex screens to purify the bacterial symbionts. ^{14}C-methane assimilation experiments with the purified symbionts have been used to verify their viability after this treatment. These viable, purified symbionts will be used in a number of experiments in the upcoming two years.

Preliminary results from both our laboratory and Dr. Felbecks at SIO indicate that N_2 may be an important source of nitrogen for this symbiosis. We have seen a consistent consumption of N_2 in our whole animal and isolated gill respiration studies and J. Stein in Felbeck's laboratory has provisionally identified a nif gene in symbiont DNA preparations using a nif structural gene as a hybridization probe.

We returned to the Louisiana Slope hydrocarbon seeps this past August and successfully collected (using the submersible Pisces II, funded through NOAA, NURP) and shipped back to our laboratory over 800 live mussels. These mussels are housed in a newly modified (with greatly expanded capacity) maintenance system. These mussels will be used in the experiments planned for the next year. We have now kept over 100 mussels (collected last June) alive in our maintenance system for over one year. Additionally, fluids from freshly collected mussels were analyzed on board ship during that cruise for methane and sulfide, and fixed for subsequent laboratory analysis of thiosulfate, as were interstitial water samples from their environment. These analyses, when combined with laboratory experiments, will be used to determine concentrations of potential metabolites and poisons in their environment.

WORK PLAN (Year 2):

Much of the work outlined below will be accomplished using the mussels we have collected this past August.

1) We will submit by the end of October, 1988 the completed study of particulate feeding by the seep mussel. The provisional title is "The role of suspension-feeding in the nutritional biology of a hydrocarbon-seep mussel" by H. M. Page, C. R. Fisher and J. J. Childress, and will be submitted to Marine Ecology Progress Series.

2) In November we will conduct 14C-methane incubations with live animals to follow quantitatively the time course of incorporation of methane into organic carbon by the symbionts, and the subsequent transfer of organic carbon from the symbionts to the host mussel. When these results are combined with the results of the autoradiographic incubations (already completed, but not analyzed), we will have considerable information on the rates of incorporation and mode of transfer of organic carbon in this symbiosis.

3) In early 1989 we will take two weeks to conduct a number of enzyme assays on tissues from several ongoing studies. This will include samples of gill tissue from the growth study outlined above. When those assays are done the growth study will be complete and will be submitted for publication by mid 1989.

4) We will continue our investigations on pathways of nitrogen assimilation by the symbiosis. These will include: Acetylene reduction experiments; further quantitative analysis of NH_4^+ and N_2 uptake by live animals; and continuation of ongoing experiments to measure the potential for uptake (and rates of uptake) of dissolved organic nitrogen sources (amino acids).

5) We will measure rates of methane, oxygen, and carbon dioxide flux in live animals under various concentrations of methane, oxygen, and sulfide to determine the dependency and sensitivity of the mussels to these substances. We will also measure the concentrations of sulfide, methane, and thiosulfate in the tissues of mussels exposed to different concentrations of methane and sulfide and compare these results to the results of the shipboard analyses of fresh animals already completed.

6) We will conduct the preliminary studies for our investigation of genetic autonomy of the symbionts. These studies will be used to determine the optimal time courses for introduction of label into the partners, the concentrations and specific activity of label to use, and the optimal concentrations of inhibitors for the planned experiments.

INVENTIONS:

none

PUBLICATIONS AND RESEARCH PRESENTATIONS:

1) Work done as a part of this study, but completed before the award arrived, was published in Science.

Cary, S. C., C. R. Fisher, and H. Felbeck (1988) Mussel growth supported by methane as sole carbon and energy source. Science, 240: 78-80.

2) A paper detailing our recent studies with this mussel was presented at the Fifth International Deep-Sea Biology Symposium in Brest, France. (July 26-June 1, 1988).

Childress, J. J. and C. R. Fisher (1988) The methanotrophic symbiosis in a hydrocarbon seep mussel.

3) A paper with the results of the particulate feeding experiments has been accepted and will be presented at the December meeting of the American Society of Zoologists in San Francisco, CA (Dec. 27-31, 1988)

Page, H. M., C. R. Fisher, and J. J. Childress (1988) Suspension-feeding and the nutritional biology of a hydrocarbon seep and a hydrothermal vent mussel.

TRAINING ACTIVITIES AND PERSONAL:

Dr. H. M. Page was employed part time for 6 months for work on Particulate feeding experiments (male, Caucasian). J Dugan was employed for 10 months for work on growth experiments (female, Caucasian). R. Kochevar, a graduate student, was also paid partially by this grant (male, Caucasian). He is working on the effects of sulfide and methane concentration on methane assimilation and respiration by the mussel.